

UNCLASSIFIED

AD NUMBER
ADB286365
NEW LIMITATION CHANGE
TO Approved for public release, distribution unlimited
FROM Distribution authorized to U.S. Gov't. agencies only; Proprietary Information; Jun 2002. Other requests shall be referred to USAMRMC, Ft. Detrick, MD 21702
AUTHORITY
USAMRMC ltr, dtd 28 July 2003

THIS PAGE IS UNCLASSIFIED

AD _____

Award Number: DAMD17-00-1-0644

TITLE: Vitamin D Receptor and Mammary Tumorigenesis

PRINCIPAL INVESTIGATOR: JoEllen Welsh, Ph.D.

CONTRACTING ORGANIZATION: University of Notre Dame
Notre Dame, Indiana 46556

REPORT DATE: June 2002

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Distribution authorized to U.S.
Government agencies only (proprietary information, Jun 02). Other
requests for this document shall be referred to U.S. Army Medical
Research and Materiel Command, 504 Scott Street, Fort Detrick,
Maryland 21702-5012.

The views, opinions and/or findings contained in this report are
those of the author(s) and should not be construed as an official
Department of the Army position, policy or decision unless so
designated by other documentation.

20030214 141

NOTICE

USING GOVERNMENT DRAWINGS, SPECIFICATIONS, OR OTHER DATA INCLUDED IN THIS DOCUMENT FOR ANY PURPOSE OTHER THAN GOVERNMENT PROCUREMENT DOES NOT IN ANY WAY OBLIGATE THE U.S. GOVERNMENT. THE FACT THAT THE GOVERNMENT FORMULATED OR SUPPLIED THE DRAWINGS, SPECIFICATIONS, OR OTHER DATA DOES NOT LICENSE THE HOLDER OR ANY OTHER PERSON OR CORPORATION; OR CONVEY ANY RIGHTS OR PERMISSION TO MANUFACTURE, USE, OR SELL ANY PATENTED INVENTION THAT MAY RELATE TO THEM.

LIMITED RIGHTS LEGEND

Award Number: DAMD17-00-1-0644
Organization: University of Notre Dame

Those portions of the technical data contained in this report marked as limited rights data shall not, without the written permission of the above contractor, be (a) released or disclosed outside the government, (b) used by the Government for manufacture or, in the case of computer software documentation, for preparing the same or similar computer software, or (c) used by a party other than the Government, except that the Government may release or disclose technical data to persons outside the Government, or permit the use of technical data by such persons, if (i) such release, disclosure, or use is necessary for emergency repair or overhaul or (ii) is a release or disclosure of technical data (other than detailed manufacturing or process data) to, or use of such data by, a foreign government that is in the interest of the Government and is required for evaluational or informational purposes, provided in either case that such release, disclosure or use is made subject to a prohibition that the person to whom the data is released or disclosed may not further use, release or disclose such data, and the contractor or subcontractor or subcontractor asserting the restriction is notified of such release, disclosure or use. This legend, together with the indications of the portions of this data which are subject to such limitations, shall be included on any reproduction hereof which includes any part of the portions subject to such limitations.

THIS TECHNICAL REPORT HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION.

Kath Mon 11/6/03

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE June 2002	3. REPORT TYPE AND DATES COVERED Final (1 Sep 00 - 31 May 02)	
4. TITLE AND SUBTITLE Vitamin D Receptor and Mammary Tumorigenesis			5. FUNDING NUMBERS DAMD17-00-1-0644	
6. AUTHOR(S) JoEllen Welsh, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Notre Dame Notre Dame, Indiana 46556 E-Mail: jwelsh3@nd.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES report contains color				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Distribution authorized to U.S. Government agencies only (proprietary information, Jun 02). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.				12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 Words) In these studies, we have tested whether VDR ablation enhances sensitivity to oncogenic transformation, using the MMTV-neu transgenic mouse model of breast cancer. Our studies involved crossing VDRKO mice with MMTV-neu mice and monitoring tumor development and mammary gland morphology over time. Tumor development in the bigenic mice was slowed due to the influence of the C57BL6 genetic background, which is known for resistance to mammary tumorigenesis. At 15 months of age, tumor development was less than 40% in bigenic mice, compared to 100% incidence in parental MMTV-neu mice at 8.5 months of age. Histological assessment of MMTV-neu primary tumors indicated no consistent effects of VDR ablation on tumor morphology or neu expression. VDR was highly expressed in neu driven mammary tumors. The delay in tumor development precluded a rigorous test of the effect of VDR ablation on MMTV-neu tumorigenesis. We therefore examined mammary gland morphology at 12 months of age and found increased incidence of pre-neoplastic lesions, as well as dilated and thickened ducts, in MMTV-neu/VDRKO mice compared to MMTV-neu/VDR WT mice. These data suggest that VDR ablation impacts on the morphology of the mammary gland in MMTV-neu mice, and that neu driven mammary tumors, which have high levels of VDR protein, might be responsive to vitamin D based therapeutics that trigger VDR signaling.				
14. SUBJECT TERMS MMTV-neu tumorigenesis, VDR				15. NUMBER OF PAGES 13
				16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Table of Contents

Cover	
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4-6
Key Research Accomplishments.....	7
Reportable Outcomes.....	7
Conclusions.....	7
References.....	7
Appendices.....	7-12

INTRODUCTION

In these studies, we are testing the hypothesis that the Vitamin D3 Receptor (VDR) represents a nutritionally modulated growth regulatory gene in mammary gland. The VDR is a nuclear receptor that modulates gene expression when complexed with its ligand, 1,25dihydroxyvitamin D3 (1,25D), the active metabolite of vitamin D3. We propose that VDR induces genes which suppress proliferation and maintain differentiation in mammary gland, and consequently, that dysregulation of VDR target genes will predispose mammary epithelial cells to transformation. Extensive preclinical and clinical data indirectly support this concept. In animal models, vitamin D3 negates the effects of dietary fat and chemical carcinogens on mammary tumorigenesis. In clinical studies, low serum 1,25D correlates with increased breast cancer risk and metastasis. Data from the NHANES I epidemiological study suggests that optimal vitamin D3 nutrition affords protection against breast cancer. Genetic studies have identified specific alleles of the VDR which correlate with an increased risk for sporadic breast cancer and with more aggressive metastatic disease. Mechanistic data indicate that 1,25D inhibits proliferation of human breast cancer cells via induction of growth arrest and apoptosis. Despite these consistent data supporting a protective role for vitamin D3 and its receptor in breast cancer, it has been difficult to demonstrate that lack of vitamin D3 directly affects mammary gland *in vivo*. Dietary vitamin D3 deficiency has profound effects on calcium homeostasis which confound interpretation of 1,25D's effect on mammary gland differentiation. In recent studies we have examined the effect of VDR ablation on mammary gland using VDR knockout (VDRKO) mice fed a high calcium "rescue" diet which normalizes calcium homeostasis (Zinser et al, 2002). These studies indicated that lack of VDR accelerates mammary gland development during puberty, supporting the concept that the VDR suppresses hormone driven proliferation in mammary cells. In the studies funded by DAMD17-00-1-0644, we have used VDRKO mice to directly examine if lack of the VDR in mammary gland enhances sensitivity to oncogenic transformation and whether mammary tumors which lack VDR exhibit more aggressive behavior than tumors that express VDR.

BODY OF REPORT

Experimental Design.

To test whether VDR ablation enhances sensitivity to oncogenic transformation, we chose a transgenic mouse model of breast cancer, the MMTV-neu mice (Guy et al, 1992). Our studies involved crossing VDRKO mice with MMTV-neu mice, which overexpress the proto-oncogene neu, a tyrosine kinase growth factor receptor in the EGFR family, and predictably develop metastatic breast cancer. Homozygous MMTV-neu mice are viable and fertile and develop focal mammary tumors beginning at 4 months of age, which are preceded by hyperplastic lesions. This model was chosen for the following reasons: 1) the latency, frequency, histology, and metastatic potential of the mammary lesions is well characterized, 2) the phenotype is not associated with very rapid onset or overly aggressive tumors; thus enabling detection of synergy if it exists; 3) synergistic tumorigenesis has been observed in crosses of MMTV-neu mice and transgenic mice overexpressing TGF α ; demonstrating the feasibility of our approach, and 4) 1,25D has been shown to oppose signaling through the EGF family of receptors; thus, lack of VDR may be expected to amplify tumorigenesis initiated by neu amplification.

Results: The data generated by these studies include assessment of tumor incidence, tumor morphology, VDR and neu expression, pre-neoplastic lesions and ductal branching in mammary gland whole mounts as described below. All Figures referenced in this section are included in the Appendix.

Generation of MMTV-neu mice with or without VDR ablation.

To generate bigenic mice, female MMTV-neu mice (purchased from Jackson Laboratories) were mated to male VDRKO mice from our breeding colony. Heterozygous offspring were crossed to generate MMTV-neu mice that were VDR WT, VDR heterozygous (VDR HET) or VDR KO. No difficulties in breeding, fertility or pup survival were noted in the crosses, and sufficient numbers of mice to test the hypothesis were generated. Three distinct groups of female mice that carried the MMTV-neu transgene were monitored in our study:

1. *Parental MMTV-neu mice, line #202*, which were those originally obtained from Jackson Labs that were bred to generate the bigenic mice. These mice were wild type for VDR and were multiparous (since they were breeders). There were 20 mice in this group.
2. *The F1 generation mice*, which were derived from crossing parental MMTV-neu mice with VDRKO mice. These mice were heterozygous for the VDR and were also multiparous, since they were used to generate the experimental (F2) mice. There were 19 mice in this group.
3. *The F2 generation mice*, derived from crossing males and females of the F1 generation. These mice differed in VDR expression: either VDR WT (n = 76), VDR HET (n=164) or VDR KO (n=120). These mice were not bred. Only female offspring were used for study.

Mammary Tumor development.

All mice were palpated weekly to detect tumors, and tumor incidence was plotted against time for all three groups of animals. Data on tumor development (expressed as percentage of tumor free mice) is presented in Figure 1.

Group 1. In the parental MMTV-neu females, tumors were detected as early as 5 months of age. In these mice, 50% tumor incidence was observed at 6.5 months of age, and all mice were tumor positive by 8.5 months of age (Figure 1A). These data on tumor development are highly comparable to that reported by Guy et al (1992) for the original strain (line #202) of MMTV-neu mice.

Group 2. The F1 generation mice were obtained by crossing MMTV-neu females (which are on the FVB genetic background) with VDRKO mice (which are on the C57BL6 genetic background). We found that the mixture of genetic backgrounds significantly slowed mammary tumor development. In these F1 MMTV-neu mice, the first tumors were detected at 6.5 months of age, and 50% tumor incidence was observed at 12.5 months of age (Figure 1B). This is not unexpected since the C57BL6 background is considered to be more resistant to mammary tumor development than other genetic backgrounds (Rowse et al, 1998).

Group 3. In F2 MMTV-neu mice, tumor development was further delayed compared to F1 MMTV-neu mice (Figure 1C), as the first tumors were detected at >8 months of age in F2 mice. We thus requested and received a no-cost extension to continue monitoring mice up to 15 months of age. The final tumor development curves were virtually identical for the three VDR genotypes up until 13 months of age. Final tumor incidence (at 15 months) was 31% (in MMTV-neu/VDR WT mice, 22% in MMTV-neu/VDRKO mice and 38% in MMTV-neu/VDR HET mice. Further statistical analysis is underway to determine if MMTV-neu driven mammary tumor development curves are significantly different as a function of VDR genotype. A confounding problem is that aging MMTV-neu/VDRKO mice were not as healthy as MMTV-neu/VDR WT mice or MMTV-neu/VDR HET mice, and this may have adversely impacted long term tumor development indirectly.

These data indicate that tumor incidence was lower in all F2 generation mice, regardless of genotype, than in F1 mice. This difference may reflect the nulliparous state of the F2 mice, as compared to F1 mice, which were multiparous, since endogenous hormones can stimulate the MMTV promoter (and thus enhance transgene expression) during pregnancy. However, other factors may also be involved, since Guy et al (1992) have reported that tumor development in line #202 (on FVB background) is not strictly dependent on pregnancy. For example, the importance of pregnancy in susceptibility to tumor development could be modified by the C57BL6 genetic background.

Analysis of mammary tumors.

Tumors were collected from F2 generation mice to assess general pathology and expression of the VDR and the neu transgene. As demonstrated in Figure 2, tumors from MMTV-neu/VDR WT mice (Figure 2A), MMTV-neu/VDR HET mice (Figure 2B), and MMTV-neu/VDRKO mice (Figure 2C) were composed of solid nests of pale intermediate cells characteristic of neu transgene expression (Guy et al, 1992). No consistent differences in tumor pathology were noted as a function of VDR genotype.

A prerequisite for an effect of VDR ablation on MMTV-neu driven tumorigenesis would be that neu tumors express VDR. Since there have been no publications regarding the expression of VDR in MMTV-neu tumors in mice, or neu expressing breast cancers in women, we examined VDR expression by immunohistochemistry in tumors derived from MMTV-neu/VDR WT mice (Figure 3A), MMTV-neu/VDR HET mice (Figure 3C), and MMTV-neu/VDRKO mice (Figure 3E). Nuclear VDR expression was detected in primary tumors derived from MMTV-neu/VDR WT mice and MMTV-neu/VDR HET mice but not in those from MMTV-neu/VDRKO mice. VDR was also detected in lung metastases from MMTV-neu/VDR WT mice and MMTV-neu/VDR HET mice (data not shown). These data indicate that therapeutics that activate VDR signaling, such as vitamin D analogs, might be effective growth inhibitors of tumors that express neu.

To verify expression of the neu transgene in these tumors, sections were subjected to immunohistochemistry with an antibody that recognizes rat c-neu. As demonstrated in Figure 3, tumors from MMTV-neu/VDR WT mice (Figure 3B), MMTV-neu/VDR HET mice (Figure 3D), and MMTV-neu/VDRKO mice (Figure 3F) expressed comparable levels of membrane localized neu protein. These results indicate that VDR ablation did not alter neu expression in the tumors.

Both frozen and paraffin embedded mammary tumor samples from MMTV-neu/VDRKO mice, MMTV-neu/VDR HET mice and MMTV-neu/VDR WT mice have been archived for future studies.

Mammary gland morphology.

The delayed development of mammary tumors in MMTV-neu mice on the C57BL6 background prompted us to examine the effect of VDR ablation on mammary gland morphology in MMTV-neu mice. At 2, 4 and 6 months of age, two-three mammary glands were surgically dissected from MMTV-neu/VDRKO mice and MMTV-neu/VDR WT mice, whole mounted and stained with carmine-alum. No pre-neoplastic or other lesions were detected in mammary glands of mice of either genotype at these ages.

A more extensive examination of mammary gland morphology was conducted in 12 non-tumor bearing F2 MMTV-neu mice of each genotype at 10 months of age. Whole mounts were scored for alveolar growth, ductal thickness and pre-neoplastic lesions by three observers in a double blind fashion. Representative whole mounts are presented in Figure 4. The left panels demonstrate ductal morphology and branching, whereas the right panels demonstrate pre-neoplastic lesions. Top panels (A,B) are from MMTV-neu/VDR WT mice, middle panels (C,D) are from MMTV-neu/VDR HET mice and lower panels (E, F) are from MMTV-neu/VDR KO mice. The data indicated that VDR genotype did not affect the extent of alveolar growth in MMTV-neu mice. However, the incidence of pre-neoplastic lesions tended to be higher in MMTV-neu/VDRKO mice (82%) and MMTV-neu/VDR HET mice (75%) than in MMTV-neu/VDR WT mice (50%). In addition, generalized thickening and dilation of the mammary ducts was present in 92% of MMTV-neu/VDR KO mice and 25% of MMTV-neu/VDR HET mice, compared to 8% of MMTV-neu/VDR WT mice. These findings indicate that VDR ablation impacts on the gross morphology of the aging mammary gland, and future studies to assess histopathology and neu expression in relation to the VDR in mammary gland are warranted.

Summary.

These studies utilized VDR ablated mice to examine the role of the vitamin D signaling pathway on neu driven tumorigenesis in a mouse model. The data indicate that neu driven tumors express the VDR and would therefore likely be amenable to vitamin D based therapeutics. In these studies, VDR ablation did not enhance the development of primary tumors, however, MMTV-neu mice lacking VDR were not as healthy and long lived as littermates expressing VDR. Tumor development may have been slightly enhanced in MMTV mice heterozygous for VDR compared to those wild type for VDR. Mammary gland morphology of MMTV-neu mice lacking VDR was characterized by thickened dilated ducts which were not found in MMTV-neu mice wild type for VDR. VDR ablation also appeared to enhance the development of pre-neoplastic ductal lesions in mammary gland. These studies implicate the vitamin D endocrine system in negative growth regulation of the mammary gland, and support the potential use of vitamin D3 analogs in prevention or treatment of breast cancer.

KEY RESEARCH ACCOMPLISHMENTS

- Generated MMTV-neu transgenic mice on the VDR KO, VDR WT or VDR Het background.
- Assessed the effect of VDR ablation on neu driven mammary tumorigenesis and pre-neoplastic lesions
- Characterized the effects of VDR ablation on mammary gland morphology of aging MMTV-neu mice
- Demonstrated the presence of the VDR in MMTV-neu tumors
- Archived mammary tumor samples from MMTV-neu/VDRKO mice, MMTV-neu/VDR HET mice and MMTV-neu/VDR WT mice for subsequent gene array, proteomic or histological analyses.

REPORTABLE OUTCOMES

Zinser GM and Welsh JE. Vitamin D receptor and mammary tumorigenesis. Abstract, Era of Hope meeting, to be presented Sept. 2002.

CONCLUSIONS

The concept tested in these studies is that nutritional regulation (dietary vitamin D3) of a putative tumor suppressor gene (the VDR) could modify breast cancer development. During the time frame of the Concept award, we generated MMTV-neu mice on the VDRKO background, and demonstrated that VDR ablation impacted on mammary gland morphology and pre-neoplastic lesion development in MMTV-neu mice. The time course of tumor development in this model did not allow for analysis of the effect of VDR ablation on actual tumor development, since the incidence of tumor formation was low even at 15 month follow-up. However, we demonstrated that tumors that developed in response to the oncogene neu express the VDR, suggesting that vitamin D derivatives that trigger VDR mediated growth inhibition might be useful in treatment of human breast tumors that express neu.

REFERENCES

Zinser GM, Packman K and Welsh JE. Vitamin D3 receptor ablation alters mammary gland morphogenesis. *Development* 129:3067-3076, 2002

Guy CT, Webster MA, Schaller M, Parsons TJ, Cardiff RD, Muller WJ. Expression of the neu protooncogene in the mammary epithelium of transgenic mice induces metastatic disease. *Proc Natl Acad Sci U S A.* 89:10578-10582, 1992

Rowse GJ, Ritland SR, Gendler SJ. Genetic modulation of neu proto-oncogene-induced mammary tumorigenesis. *Cancer Res.* 58:2675-2679, 1998

APPENDICES

Figure 1. Tumor incidence in MMTV-neu mice

Figure 2. MMTV-neu tumor morphology

Figure 3. VDR and neu expression in MMTV-neu tumors

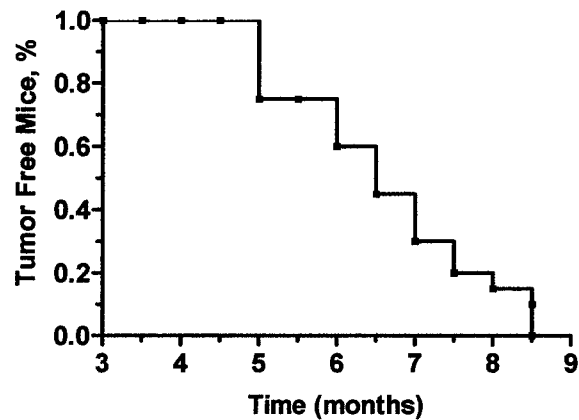
Figure 4. Representative whole mounts from MMTV-neu mice

Abstract to be presented at Era of Hope meeting, September, 2002.

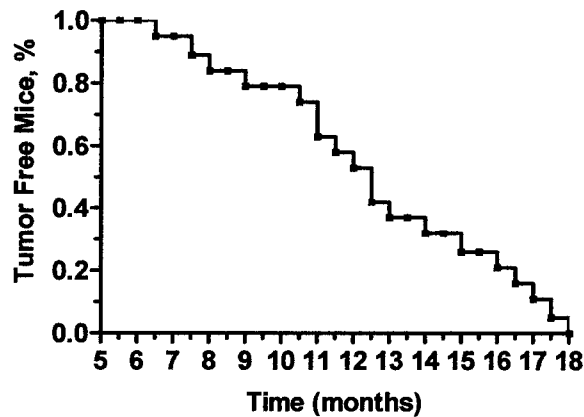
Zinser GM and Welsh JE. Vitamin D receptor and mammary tumorigenesis.

Figure 1. Tumor incidence vs. time in parental (A), F1 generation (B) and F2 generation (C) MMTV-neu mice.

A. Parental MMTV-neu mice (VDR WT)



B. F1 generation MMTV-neu mice (VDR Het)



C. F2 generation MMTV-neu mice (VDR WT, VDR Het and VDRKO)

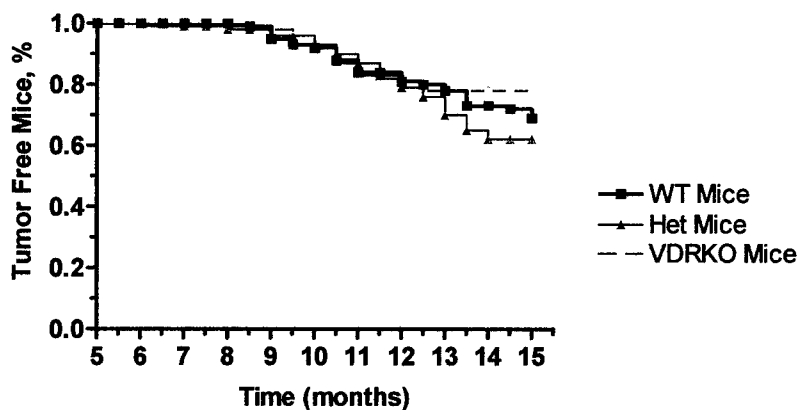


Figure 2. Pathology of mammary tumors from F2 generation mice. H&E staining was performed on tumor sections from F2 generation MMTV-neu mice. A: VDR WT; B: VDR Het; C: VDR KO

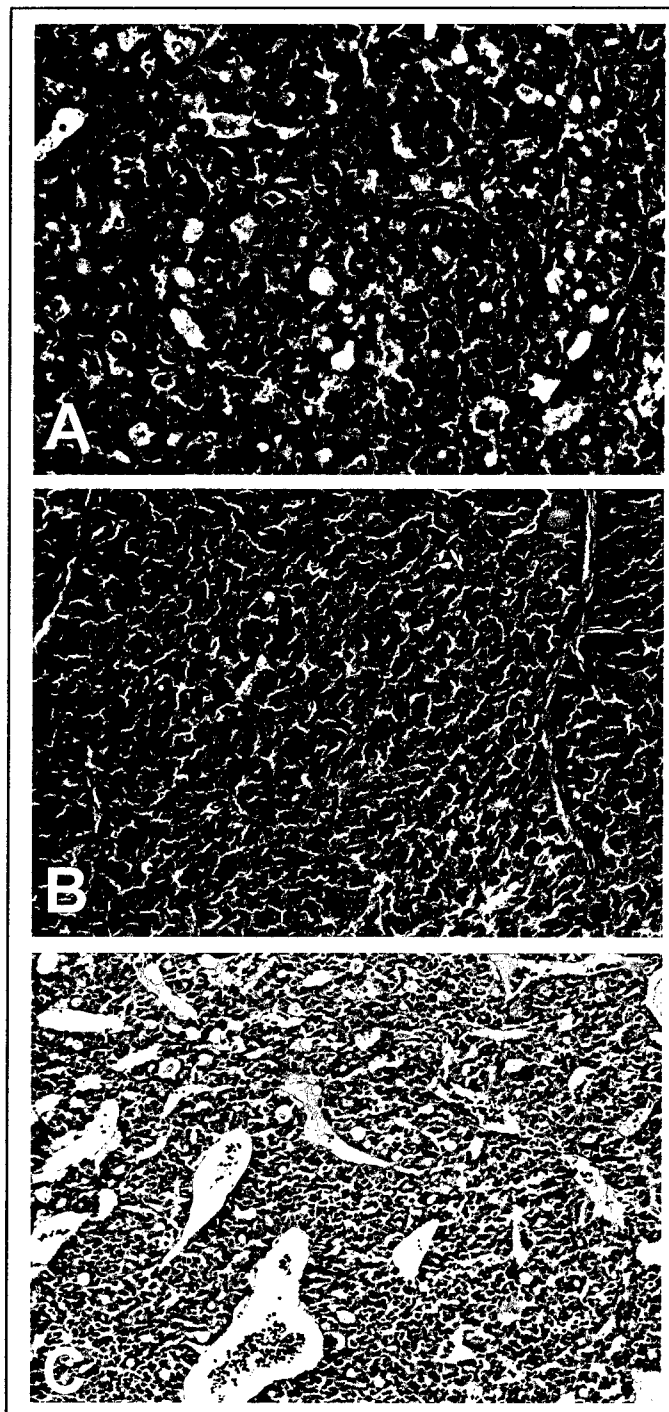


Figure 3. VDR and Neu expression in mammary tumors from F2 generation mice. Immunohistochemistry for VDR (A,C,E) and c-Neu (B,D,F) was performed on tumor sections from F2 generation MMTV-neu mice. A,B: VDR WT; C,D: VDR Het; E,F: VDR KO

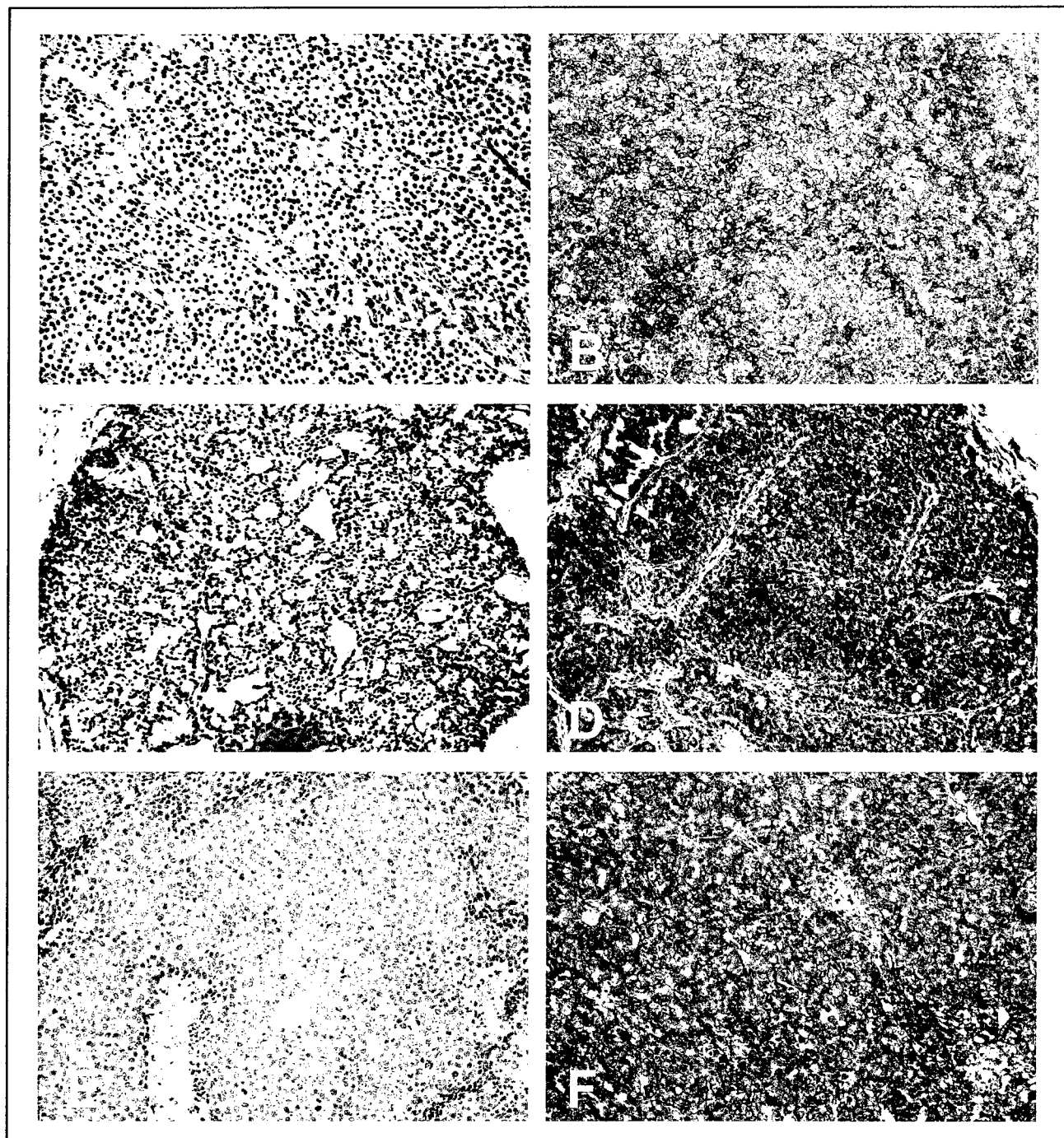
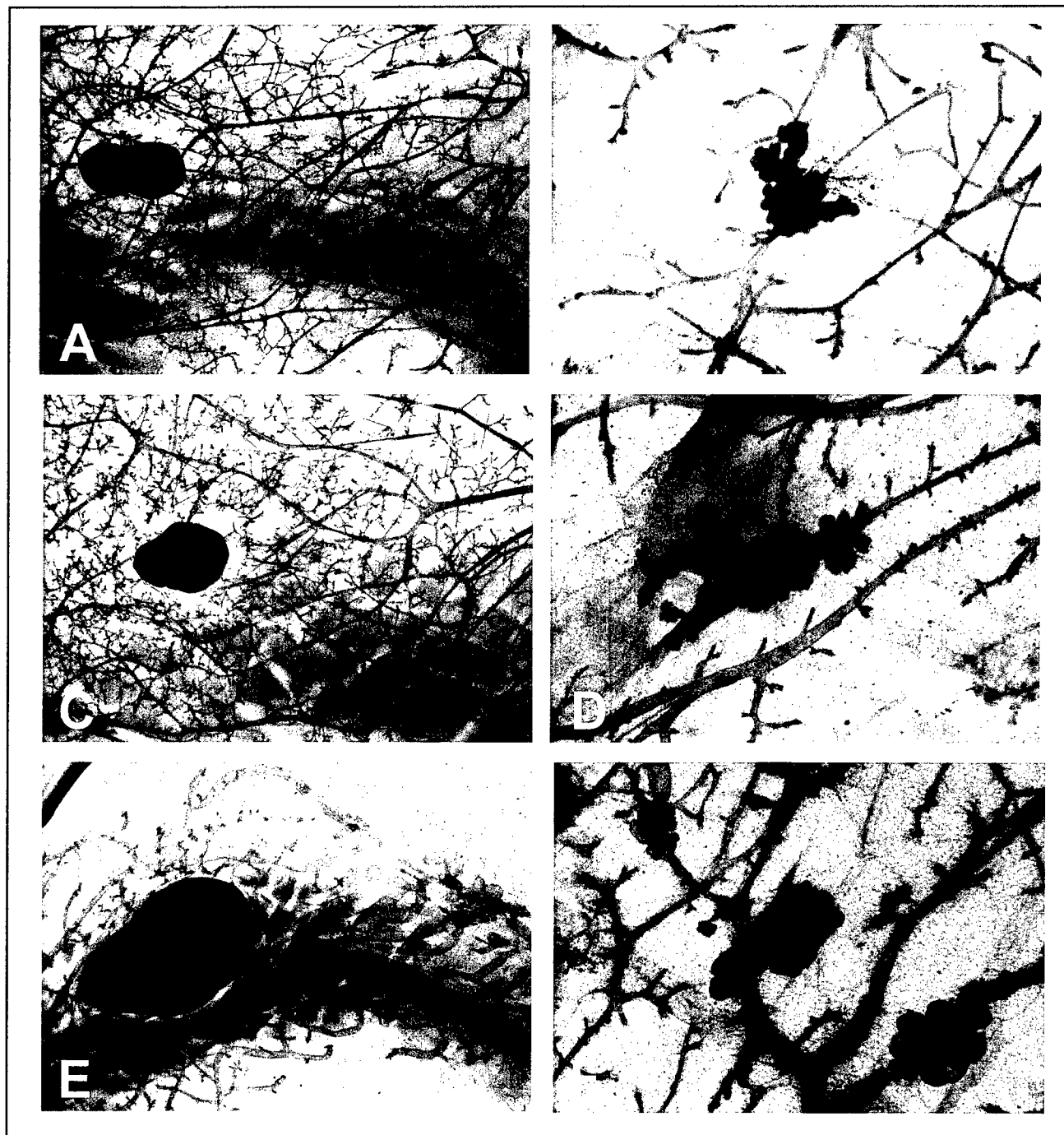


Figure 4. Whole mounts of F2 generation mice. Ductal architecture (A,C,E) and pre-neoplastic lesions (B,D,F) of F2 generation MMTV-neu mice. A,B: VDR WT; C,D: VDR Het; E,F: VDR KO



VITAMIN D RECEPTOR AND MAMMARY TUMORIGENESIS

Glendon M. Zinser and JoEllen Welsh

University of Notre Dame, Department of Biology

jwelsh3@nd.edu

In these studies, we are using the vitamin D3 receptor knockout (VDR KO) mouse to test the hypothesis that the VDR represents a tumor suppressor gene in mammary gland. In VDR KO mice, we have observed accelerated mammary gland development during puberty, suggesting that the VDR suppresses hormone driven proliferation in mammary cells. To test whether VDR ablation enhances sensitivity to oncogenic transformation, VDR KO males were mated with MMTV-neu females, and heterozygous offspring were crossed to generate MMTV-neu mice that were VDR WT, VDR heterozygous (VDR HET) or VDR KO. Tumor development was followed in multiparous parental MMTV-neu mice (VDR WT; n = 20), multiparous F1 MMTV-neu mice (VDR HET, n = 19) and in virgin F2 MMTV-neu mice [VDR WT (n = 76), VDR HET (n=164) or VDR KO (n=120)]. In parental MMTV-neu females, 50% tumor incidence was observed at 6.5 months of age. Crossing MMTV-neu females (on FVB background) with VDR KO mice (on C57BL6 background) slowed tumor development, with 50% tumor incidence observed in F1 MMTV-neu mice at 12 months of age. In F2 MMTV-neu nulliparous mice, tumor development was further delayed, with less than 20% incidence for all genotypes at 11 months of age (continued follow-up is in progress). The delayed development of mammary tumors on the C57BL6 background prompted us to examine mammary gland morphology in 12 virgin F2 MMTV-neu mice of each genotype at 10 months of age. We found that although VDR genotype did not affect alveolar growth, the incidence of pre-neoplastic lesions tended to be higher in VDR KO mice (82%) and VDR HET mice (75%) than in VDR WT mice (50%). In addition, generalized thickening and dilation of the mammary ducts was present in 92% of VDR KO mice and 25% of VDR HET mice, compared to 8% of VDR WT mice. These findings indicate that VDR ablation impacts on the gross morphology of the aging mammary gland, and further studies to assess histopathology and neu expression in relation to the VDR are in progress. In summary, our studies in VDR ablated mice implicate the vitamin D3 endocrine system in negative growth regulation of the mammary gland, and support the potential use of vitamin D3 analogs in prevention or treatment of breast cancer.



DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y)

28 July 03

MEMORANDUM FOR Administrator, Defense Technical Information
Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir,
VA 22060-6213


SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

Encl


PHYLLIS M. RINEHART
Deputy Chief of Staff for
Information Management

ADB233865	ADB264750
ADB265530	ADB282776
ADB244706	ADB286264
ADB285843	ADB260563
ADB240902	ADB277918
ADB264038	ADB286365
ADB285885	ADB275327
ADB274458	ADB286736
ADB285735	ADB286137
ADB286597	ADB286146
ADB285707	ADB286100
ADB274521	ADB286266
ADB259955	ADB286308
ADB274793	ADB285832
ADB285914	
ADB260288	
ADB254419	
ADB282347	
ADB286860	
ADB262052	
ADB286348	
ADB264839	
ADB275123	
ADB286590	
ADB264002	
ADB281670	
ADB281622	
ADB263720	
ADB285876	
ADB262660	
ADB282191	
ADB283518	
ADB285797	
ADB269339	
ADB264584	
ADB282777	
ADB286185	
ADB262261	
ADB282896	
ADB286247	
ADB286127	
ADB274629	
ADB284370	
ADB264652	
ADB281790	
ADB286578	